ELECTRONICALLY FILED ON **March 24, 2008**		
DECLARATION OF **KEN Y. LIN** **UNDER 37 C.F.R. § 1.132**	Attorney Docket Confirmation No.	STAN-276 9855
	First Named Inventor	Ken Y. Lin
	Application Number	10/713,674
	Filing Date	November 13, 2003
	Group Art Unit	1641
Address to: Commissioner for Patents Alexandria, VA 22313-1450	Examiner Name	D.J. Venci
	Title	*Methods for detecting asymmetric dimethylarginine in a biological sample*

Dear Sir:

1. I, Ken Y. Lin, declare and say I am a co-inventor of the claims of the above-identified patent application. I hold a B.S. degree in Biological Sciences from The Leland Stanford Junior University. I am currently enrolled in the MD-PhD program at Harvard Medical School with Medical Scientist Training Program fellowship from the National Institute of Health. My graduate department is biophysics.

2. I have read the Office Action dated September 24, 2007 in this application and understand that the Examiner has rejected pending claims 1-9, 15, and 17-19. The Examiner appears to be of the position that reactions of symmetric dimethylarginine (SDMA), and arginine with α-dicarbonyl compounds are unpredictable. The Examiner appears to be of the position that α-dicarbonyl compounds react with asymmetric dimethylarginine (ADMA), and that such reactions would adversely affect the claimed method.

3. The Office Action cited Baburaj et al. ((1994) *Biochim. Biophys. Acta* 1199:253; "Baburaj"), and stated that Baburaj describes two α-dicarbonyl compounds, designated "HOCGO" and "DMACGO," that are capable of reacting with cysteine, lysine, and hydrophobic surfaces.

4. Baburaj discusses the use of the α-dicarbonyl compounds HOCGO and DMACGO as pH-, polarity-, and quencher-sensitive fluorescent reporters for proteins that can be targeted at reactive arginines. The possibility that an α-dicarbonyl compound might modify a cysteine or a lysine residue would not be expected to adversely affect any of the steps of the claimed method. Indeed, Baburaj characterizes possible reactions with Cys and Lys as "a rare eventuality that can be ignored." Baburaj, page 262, column 2, second paragraph.

4. The Office Action cited Schwarzenbolz et al. ((1997) *Z. Lebensm. Unters. Forsch. A* 205:121-124; "Schwarzenbolz") and stated that Schwarzenbolz notes that under certain reaction conditions, the α-dicarbonyl compound glyoxal produces two arginine derivatives.

5. Schwarzenbolz discusses the reaction of glyoxal with arginine, and minor products that may be formed by the reaction of glyoxal with arginine. Many chemical reactions will produce, in addition to a main product, one or more side products. The side products discussed in Schwarzenbolz are minor. Any side products that may be produced in such low quantities would not be expected to adversely affect any of the steps of the claimed method.

6. The Office Action cited Sopio and Lederer ((1995) *Z. Lebensm. Unters. Forsch.* 201:381-386; "Sopio"), and stated that Sopio teaches that, under certain experimental conditions, the α-dicarbonyl compound deoxyosones, results in two tautomeric products.

7. Sopio discusses reaction of 3-deoxypentosulose with *N*-methyl- and *N,N*-dimethylguanidine as model reagents for protein-bound arginine and for creatine. Many chemical reactions will produce, in addition to a main product, one or more side products. The side products discussed in Sopio are minor. Any side products that may be produced in such low quantities would not be expected to adversely affect any of the steps of the claimed method.

8. The Office Action cited Cooper and Meister ((1978) *J. Biol. Chem.* 253:5407; "Cooper"), and stated that η-nitrogens are not required for guanidino reactivity with α-dicarbonyl compounds.

9. It is well-established that glutamate and aspartate are the two major amino acids that

undergo the cycle of trans-amination /alpha-keto acid formation, in order to bulk-transfer nitrogen waste from the peripheral tissue to the liver, where the cycle occurs in reverse to help drive the Kreb cycle. Theoretically, arginine and citrulline can go through the cycles themselves too to become a cyclic compound; however, there is no evidence to date that shows this actually happens in vivo to a significant degree. Cooper states that, under certain pathologic conditions, homocitrulline can be elevated to the point where these alpha-keto derivatives were observed in the kidneys (please see Cooper, page 5408, second paragraph under "Discussion."). However, Cooper also acknowledged that these conditions are rare point mutation genetic diseases that causes very rare metabolic dysfunction such that reaction intermediates start to pile up. In fact, mutations affecting enzymes involved in intermediate metabolism are present in less than one in a fifty thousand at the population level.

10. Furthermore, homocitrulline is not relevant to the claimed method. Homocitrulline and homoarginine are both intermediates in the amino acid pathway that are present in much lower quantities than citrulline and arginine. (The "homo" prefix means that there is an extra $-CH_2-$ moiety added to the R group of the amino acid). In summary, the reaction discussed in Cooper represents a rare derivation of arginine and citrulline, and any quantitative contribution to the method as claimed is minimal at best.

11. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

_____ _____

Date Ken Y. Lin

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